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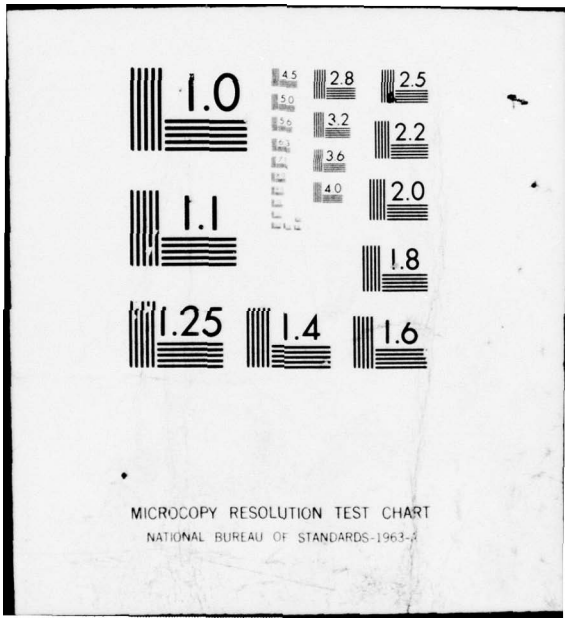
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U.S. ARMY MEDICAL INTELLIGENCE AND INFORMATION AGENCY
WASHINGTON, D.C. 20314

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USAMIIA TRANSLATION

Number: (14) USAMIIA-K-8161

Date Completed: (11) 5 May 1977

(12) 34p.

Language: Italian

Geographic Area:

English Title: (6) COMPARATIVE RESEARCH ON THE DISTRIBUTION OF THE MELANIN OF THE STRIA VASCULARIS IN THE DIFFERENT SECTIONS OF HUMAN COCHLEA. FUNCTIONAL INTERPRETATION REGARDING THE STRUCTURE OF MELANIN

Foreign Title:

Author: (10) P. Bonaccorsi

Source Document: Boll. Mal. Orrech., 83

Pages Translated: pp 829-868

Publisher: Otorhinolaryngoliatric Clinic of the University of Pavia

Date/Place Publication: 1965, Italy

Distribution Statement: Document is in the public domain

COMPARATIVE RESEARCH ON THE DISTRIBUTION OF THE MELANIN OF THE STRIA VASCULARIS IN THE DIFFERENT SECTIONS OF HUMAN COCHLEA

/829*

FUNCTIONAL INTERPRETATION REGARDING THE STRUCTURE OF MELANIN

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Director: Professor M. Cherubino)

The melanin "present in the internal ear" has always been described as a biologically inert pigment by otologists of the end of the last century (Corti, Lucae, Ranvier) and the beginning of the 20th century (Alexander, Siebenmann-Bing, Kolmer, Wolff) seeking to explain the nature of the black pigment present especially in the stria vascularis in the planum semilunatum, at the base of the macula sacculi and utriculus. They were concerned with reasons for its presence in these seats and its possible function.

The subsequent histochemical investigations (Zorzoli and Verneroni, Beck, Catalano and Madonia) as well as the recent intravital research (Cherubino, Bonaccorsi, Galioto) have allowed its classification as melanin (Figure 1).

However, almost all authors attribute no particular functional significance to this substance, the concept prevailing being that it involves random locations owing their origin to the derivation of the internal ear from the ectoderm. This could justify the presence of pigmented cellular elements of the melanogenetic system, with origin in the neural crest as well as in seats not exposed to light.

There are very few researchers who have studied this pigment or the cells containing it from a functional viewpoint.

In reality, there are only a few names listed in the bibliography and success of investigations has always been on the negative side (Beck, Catalano and Madonia).

The only test data in this field are those coming from research carried out by our clinic in the last few years and still going on. Initially, we noted a close correlation between the presence of the cells of the melanogenetic system and the vasal plexi assigned for the production or reabsorption of liquids in the animal organisms, especially in mammals and /831

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* Numbers in the right margin indicate pagination in the original text.

**Boll. Mal. Orrech., 83, 1965, pp. 829-868.
We should like to express our appreciation for the valuable collaboration by Prof. Lanzi from the Semiconductor-Free Radical Section, Prof. Mascheretti from the Hydrodynamic Section of the Institute of Physics of the University of Pavia, and Prof. Sacchi of the Dermatological Clinic of the University of Pavia.

amphibians. This is true for the choroidea of the eye, the cerebral choroidal plexi, the stria vascularis, the planum semilunatum and the bases of the macula, utriculus, sacculus and other seats. Its presence, in spite of the common ectodermal derivation of such organs did not appear to be random. It was not explained why such a distribution was present, for example, in the stria and in the planum semilunatum and absent in other seats of the anterior and posterior labyrinth. The same was true for a number of areas in the eye, brain and other organs which were also derived from the same embryonic layer.

The hypothesis was suggested that the presence of such cells was closely connected with the functions of such formations.

FIG. 1 - A

Microfotografia intravitale di coclea di cavia normocromica con breccia aperta in corrispondenza del legamento spirale del terzo giro: sono chiaramente visibili le cellule pigmentate della stria vascolare, rappresentate da elementi più voluminosi, in numero limitato e da molti altri più piccoli a disposizione prevalentemente perivasale. La biomicroscopia è stata eseguita senza alcun colorante intravitale: il contrasto, quindi, è dovuto esclusivamente al colore scuro della melanina intracellulare.

Figure 1A. Intravital microphotograph of the cochlea of the normochromic guinea pig with opening made in correspondence with the spiral ligament of the third tract. Clearly visible are the pigmented cells of the stria vascularis, represented by small-sized elements, limited in number and by many other smaller ones mostly with perivasal distribution. The biomicroscopy was always carried out without any intravital dye. The contrast then is exclusively owing to the dark color of the intracellular melanin.

/830

FIG. 1 - B

Microfotografia intravitale di coclea di cavia albina: la breccia è aperta, come la precedente, nel terzo giro, a ridosso del legamento spirale. Appare evidente la differenza tra la stria vascolare apigmentata di questa cavia albina e la stria vascolare della cavia normocromica disseminata di cellule del sistema melanogenetico, normopigmentate.

(*da:* CHERUBINO, BONACCORSI, GALIOTO)

Figure 1B. Intravital microphotograph of the cochlea of an albino guinea pig. The opening has been made like the preceding one in the third tract, very near the spiral ligament. The difference is clear between the apigmented stria vascularis of this albino guinea pig and the stria vascularis of the normochromic guinea pig having a standard distribution of melanogenetic, normal pigmented system of cells.

(By: Cherubino, Bonaccorsi, Galioto)

For this reason, we undertook a comparative research which has already been published on albino and normochromic rabbits using sodium fluorescein and we found that the various barriers such as the hemato-ophthalmic (aqueous humor, vitreous humor) the hemoliquoral and hemo-labyrinthic ones of the albinos showed a higher threshold of passage for the dye with respect to normochromic animals. From this, it was derived that the melanin or its precursors could influence capillary permeability.

However, the experiment was limited to an electronegative dye which was quite diffusible such as fluorescein and it was not given that such behavior could be extended equally to all electropositive or less diffusable chemical substances to the extent that, in the biological phenomena of membranes, it has a considerable importance apart from the molecular volume, the electrical sign of the plasma ions and their cellular endoplasmas.

We were then able, using the ample equipment found in our research laboratories, and especially owing to the placing in operation of a powerful microscope for intravital study, to successfully collect and document the special morphological patterns relating to the pigmented cells of the internal ear of rana esculenta and guinea pigs as well as a number of functional aspects of such elements induced by various stimuli. We were thus convinced that we were faced with a truly new problem for otology making necessary a later multidirectional study for the solution of many cochlea-vestibular questions.

We reported (Cherubino, Bonaccorsi, Galioto) to the Second National Congress of Microcirculation at Como last October concerning a number of results of our investigations but strictly connected with the vasal system /833
of the internal ear to which we make reference (Figures 2 and 3).

Nevertheless, even before gaining this morphofunctional knowledge, we began to collect human material (petrous bones) for a histological study on the distribution of pigmented cells of the stria vascularis by a comparative analysis with the distribution of the melanin encountered by Beck in the stria vascularis of guinea pigs and in other investigations.

This author, indeed, had emphasized how this pigment would be found in greater concentration in the apical section of the cochlea and that the latter gradually became smaller in the intermediate sections except for the base one where it was completely lacking. No particular significance was attributed to this information, referring to it only as merely morphological-topographical information.

During the intravital investigation on the microcirculation of the internal ear of guinea pigs which we conducted in these last few years (Cherubino, Galioto, Bonaccorsi, Sambuco), we were able to check this data and confirm its accuracy.

For all research carried out on this pigment, we were always seeking to find wherever possible a functional significance of many morphotopographical aspects, even when apparently intuitive in nature, which has always justified comparative research in this direction even on human beings by having available anatomohistological data on which, if necessary, we formulate interpretative hypotheses or lay down the beginnings of later investigations.

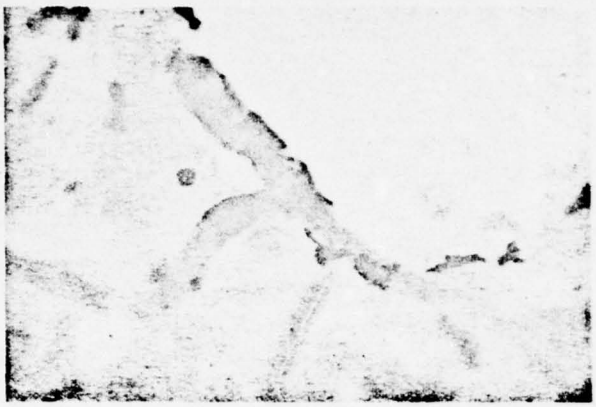

FIG. 2 - A

Rana esculenta: microfotografia intravitale della membrana perilinfatica dell'orecchio interno, che dimostra la caratteristica disposizione a manicotto in corrispondenza delle suddivisioni vasali. Sono visibili più cellule pigmentate, in fase funzionale diversa, contrastate esclusivamente per il loro contenuto in melanina; se ne nota una, non ancora perivasale, che avendo gli pseudopodi retratti, assume forma rotondeggiante, mentre le altre, già intorno ai vasi, in « atteggiamento pericitico », hanno aspetto aracniforme.

Figure 2A. Rana esculenta: Intravital microphotograph of the perilymphatic membrane of the internal ear showing the characteristic sleeve arrangement in correspondence with the vasal subdivisions. There can be seen more pigmented cells, in various functional phases, exclusively contrasted by their melanin content. One cell can be seen which is not yet perivasal which, having the pseudopods retracted, assumes a rounded shape, whereas the others already surrounding the vessels in "pericytic position" have a spider-like aspect.

/832

FIG. 2 - B

Rana esculenta: microfotografia intravitale dove appare chiaramente come i melanociti « di tipo dermico », in « atteggiamento pericitico », nelle curvature vasali si dispongano sulla superficie convessa. Anche in questo caso, come nei precedenti, non è stata fatta alcuna colorazione.

(*da:* CHERUBINO, BONACCORSI, GALIOTO)

Figure 2B. Rana esculenta: Intravital microphotograph where clearly can be seen the melanocytes "of the dermal type" in "pericytic position" in the vessel curvature being arranged on the convex surface. In addition, in this case as in the preceding ones, no dyeing was done.

(By: Cherubino, Bonaccorsi, Galioto)

The investigation appeared on the whole to be advantageous since we had come to know particulars involving the molecular structure of melanin and above all the presence of free radicals which modified the traditional concepts of the terms and conditions of protection of this pigment, not only in the skin but also in other sites of the organism where apparently the cells of the melanogenetic system appeared to only have an afunctional embryogenetic significance owing to location outside of the layers which could be reached by ultraviolet rays.

It is now advisable to note that the melanocytic endoatmia is closely connected to the melanogenesis. Indeed, the conventional Raper's diagram (Figure 4) consists, in the passage from tyrosine to indolo-5-6-quinone, essentially of three dehydrogenating processes and an oxidative decarboxylation. The same last (and more vaguely seen) phases of polymerization

with oxydo-reductive condensation of the indol-quinone in position 2-3 (and even 1) appeared to be more than ever accompanied by dehydrogenating reactions.

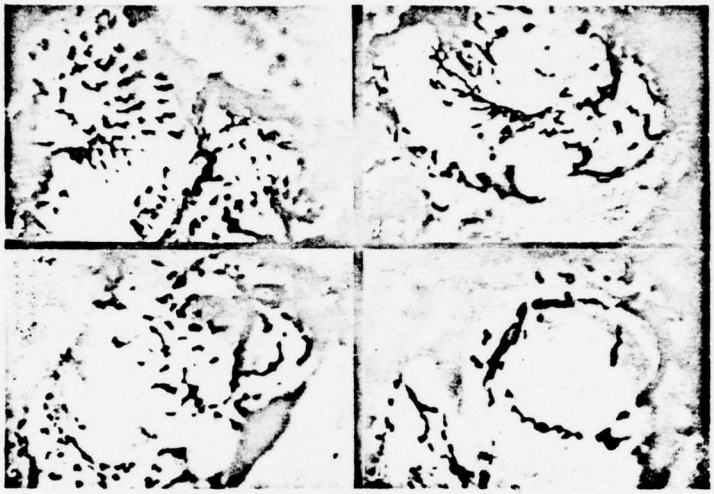

FIG. 3

Serie di fotografie intravitali di membrana perilinfatica dell'orecchio interno di rana esculenta eseguite topograficamente in uguali sedi, che presentano reti vasali molto simili, ma su esemplari diversi e in tempi successivi, per dimostrare il comportamento delle cellule pigmentate dopo esposizione degli animali a rumore intenso, in ambiente supersonorizzato industriale. È ben visibile la progressiva migrazione verso i vasi da parte dei melanociti, che dalla normale disposizione iniziale, abbastanza regolarmente distribuita (A), attraverso fasi intermedie, si accumulano a guisa di anello (B) lasciando spazi otticamente vuoti, raggiungendo nelle fasi successive (C - D) in vasi contigui, che avvolgono quasi completamente in un « mantello cellulare » di colore nero: « atteggiamento pericitico ».

(*da*: CHERUBINO, BONACCORSI, GALIOTO)

Figure 3. Series of intravital photographs of perilymphatic membrane of the internal ear of rana esculenta performed topographically in equal segments showing the vessel networks which are quite similar. Various examples and successive times are used in order to demonstrate the behavior of the pigmented cell after exposure of the animals to intense sound in a supersonified industrial environment. The progressive migration toward the vessels on the part of the melanocytes can be clearly seen differing from the normal initial arrangement. They are rather uniformly distributed (A) passing through intermediate phases, accumulating in a ring shape (B) leaving optically empty spaces, connecting in successive phases (C, D) in contiguous vessels which almost completely are surrounded in a "cellular layer" which is black in color: "pericyte attitude".

(By: Cherubino, Bonaccorsi, Galioto)

Furthermore, an active cytochrome-oxidase was located in the melanocyte on the basis of the positiveness of the reaction.

In the process of the swift and comprehensive development of electronic microscopy both with material collected using homogenization and differential centrifuging as well as with ultramicrotomic sections, there has become established a current of investigation on the ultrastructure of melanin grains.

Whereas former research empirically discussed the melanin grain as well as melanin considered purely as a unique and well-defined substance, the existence of a grain having its structure revealed that the so-called "melanin grain" had a very complex formation (Figures 5 and 6).

The complexity of the grain, explained quite well by Fitzpatrick, et al. and their "melanosome" concept shows that we are not dealing with inert particles but with metabolically active units containing a heterogeneous mixture of proteins, melanoproteins, tirosinases, dehydrogenases /835
and cytochromo-oxydases. Independently from the multiple substances through which, probably in accordance with various reaction diagrams including the conventional one of Raper (Sacchi), may be added the polymeri- /838
zation of melanin occurring from the repeated joining of adjacent molecules of indolo-5-6-quinone which involve the position 4 of one molecule and position 7 of another (Figure 7) resulting in complex chains of various lengths and in a wide spatial distribution, structurally acting to absorb "light" over a wide range of wavelengths. Hence, this is in the

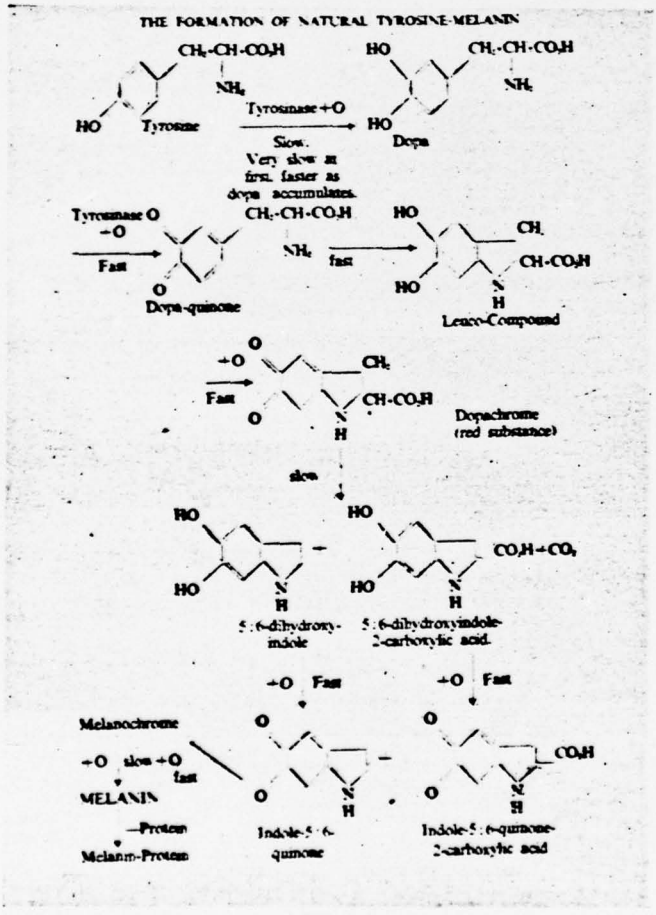

FIG. 4
Reazioni enzimatiche della catena melanogenetica, secondo Raper.
(*da:* MUNDAY e KERKUT)

Figure 4.

Enzymatic reactions of the melanogenetic chain according to Raper.

(By: Munday and Kerkut)

direction of extensive electromagnetic radiations "well beyond the limits of the visible spectrum" from ultraviolet to infrared, to Roentgen rays, gamma rays and cosmic rays which represent the essential energy source from which is conditioned and plasmaed the existence of biological life.

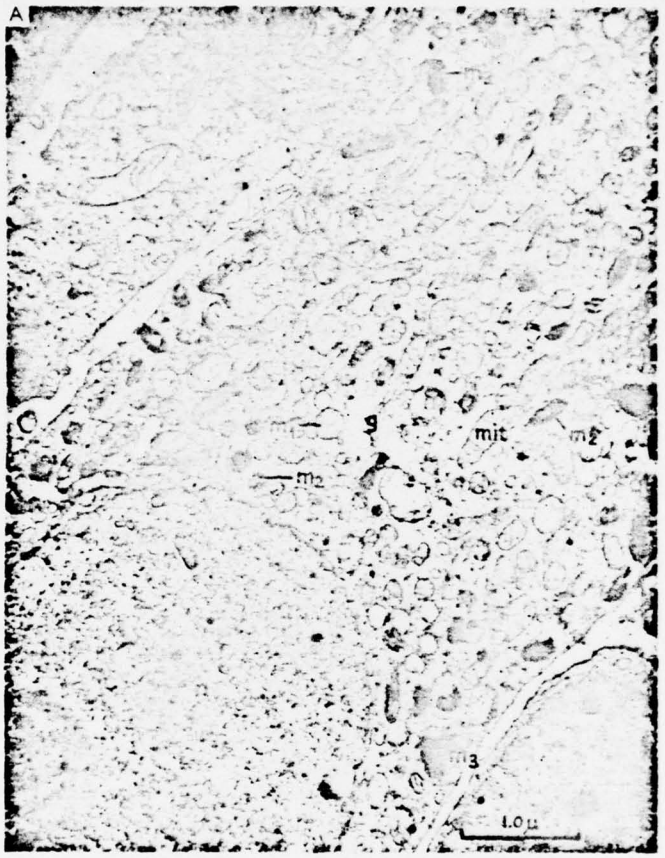

FIG. 5

Parte di un melanocita di un bulbo pilifero umano. Si vede parte del nucleo (n), e, adiacente a questo, la zona di Golgi (g) della cellula. In questa zona ci sono molte piccole vescicole che si crede siano precursori di melanosomi. In questa zona si trovano anche melanosomi prematuri (m1); questi sono sferici, ma in essi si possono vedere tracce di struttura interna. Si possono sezionare sia longitudinalmente che ortogonalmente melanosomi più maturi (m_2), nei quali la melanina si sta sintetizzando. I granuli completamente maturi (m_3) sono completamente riempiti di melanina opaca agli elettroni. Si possono anche vedere numerosi mitocondri (mit.). Bilateralmente al melanocita si vedono due cheratinociti. Queste cellule contengono molte particelle ribonucleoproteiche libere. Lo spazio fra il melanocita e il cheratinocita è tipico. (Ingrandimento x 32.000).

(*da:* BIRBECK)

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Figure 5. Part of a melanocyte of a human hair root. There can be seen part of the nucleus (n) and, adjacent to this, the Golgi zone (g) of the cell. In this zone are many small vesicles which are believed to be the precursors of melanosomes. In this zone are also found premature melanosomes (m). The latter are sperical but they allow traces of the internal structure to be seen. The more mature melanosomes (m_2) in which the melanin is synthesized can be sectioned either longitudinally or orthogonally. The completely

mature grains (m_3) are completely filled with melanin which is opaque to electrons. It is also possible to see a great many mitochondria (mit.). On both sides of the melanocyte can be seen two keratinocytes. These cells contain many free ribonucleoprotaic particles. The space between the melanocyte and the keratinocyte is typical. (Magnification 32,000 X)

(By: Birbeck)

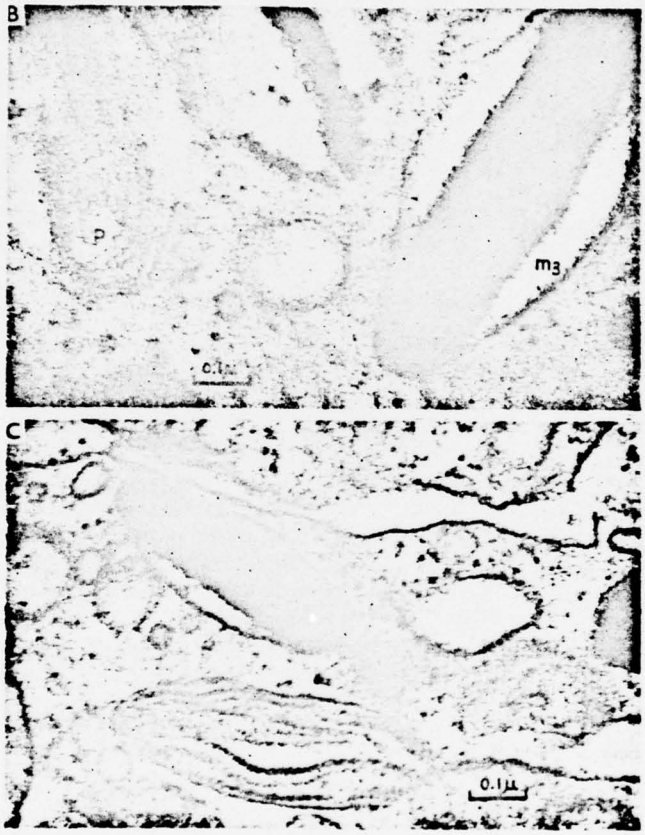

FIG. 6

B - Un premelanosoma (p), sezionato in una direzione tale che si possa vedere l'apparato delle particelle su una delle membrane interne. A destra c'è un melanosoma completamente maturo (m_3).

C - Due melanosomi intermedi che mostrano la formazione di melanina entro il granulo. Il melanosoma più in basso mostra le membrane longitudinali tagliate ortogonalmente: pochissima melanina è stata sintetizzata. Il melanosoma più alto è forse pieno a metà di melanina: si può vedere un piccolo spazio più leggero, chiaro, nel centro di ciascuna lamina di melanina che rappresenta la struttura premelanosomica originale (Ingrandimento x 160.000).

(*da:* BIRBECK)

B. One premelanosoma (p), sectioned in a direction such that it is possible to see the apparatus of the particle on one of the internal membranes. On the righthand side is a completely mature melanosome (m_3). /837

C. Two intermediate melanosomes showing the formation of melanin between the grain. The melanosome seen lower down shows the longitudinal membrane cut orthogonally. Very little melanin has been synthesized. The melanosome farther up is probably half-filled with melanin. It is possible to see a small space which is lighter and clearer in the

center of each melanin layer representing the original premelanosomic structure (magnification 160,000 X).

(By: Birbeck)

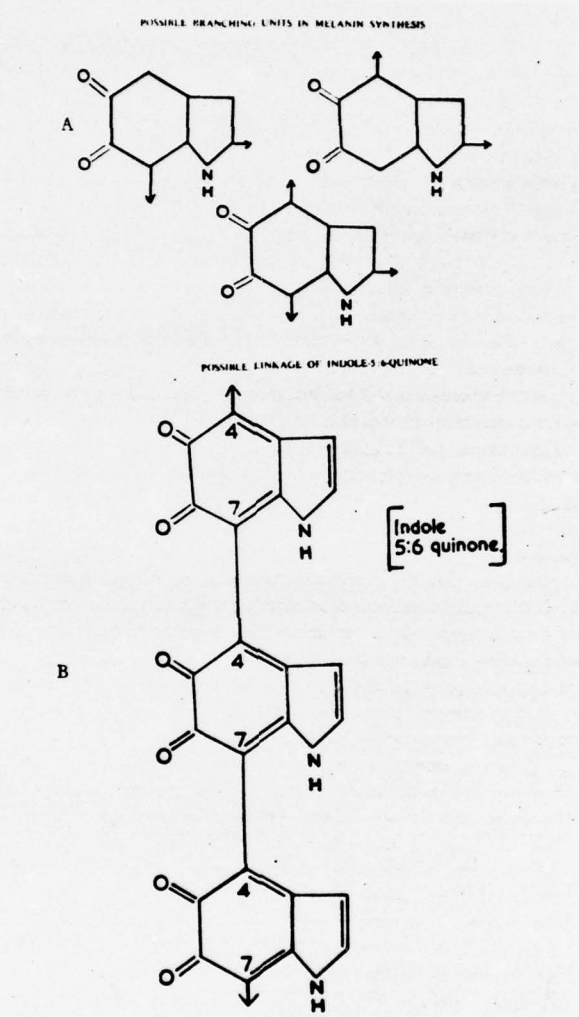

FIG. 7 - A-B - Schemi di polimerizzazione della melanina (da MUNDAY e KERKUT).

Figure 7A-B. Polymerization diagrams of melanin (by Munday and Kerkut). /839

The activity, nevertheless, of the melanin in the stage of substance which has arrived at maturity is demonstrated to the contrary of what was originally believed by the presence of free radicals in its molecule.

Commoner, Townsend and Pake were the first ones to write concerning the presence of free radicals in the melanin. The latter are found to be highly stabilized in the pigmented eggs of frogs which indicates the presence in the melanin of free electrons.

Free radicals have also been discovered in the pigment formed from enzymatic self-oxidation of "dopa" (Adams, Blais and Sands).

Mason, Ingram and Allen have made a general report on the property of free radicals of melanin derived from various animal origins even including melanin prepared from "dopa" by the action of tyrosinase from purified fungi. The property of the free radicals is attributed to a semiquinoid formation of the polymeric 5-6-indolo quinone unit which becomes stabilized by the resonance of the whole conjugated polymer.

During the enzymatic reactions which led to the formation of melanin, no trace of free radicals was found. Therefore, it was concluded that the property of melanin to have free radicals is characteristic of pigment at the stage of complete maturation.

Furthermore, investigations on the reactions of oxydo-reduction of melanin indicate that the pigment can act as cation exchange material (White).

The melanin is reduced in a reversible process by sodium hydrosulfite to a chestnut and reddish color and then, using potassium ferrocyanide, into a dark color (Figge).

The interesting observation made by Mason, Ingram and Allen that the melanin of cuttle fish ink reduced with ascorbic acid or H_2S loses much of its character of free radical reinforces the position that this is capable of forming a reversible oxydo-reduction system by means of losing or gaining electrons.

On this subject, it is interesting to note that Cassidy has shown /840
that a number of polymers in which exchange of cations occurs can absorb electrons.

The theory of semiconductivity, as means for transferring electrons (and the energy accompanying it) in the biological systems, was set forth by Szent Gyoergy. The latter considered the possibility of biological semiconductors inhibiting the function by capturing electrons or by giving up electrons thus saturating the system and making it inactive. In other cases, a reaction could be originated by the passage of an electron to a reactive point.

Now. Longuet and Higgins assumed that melanin acts as a single-dimensioned semiconductor, the condution bonds being formed by the interaction between the vacant orbits lower down and the single quinoid unit.

The oxidation of melanin leads to the production of free orbits creating positive spaces which could lead to paramagnetic molecular types.

The quantitative valuation of the free radicals is accomplished by means of special ESR spectrometers, detecting the patterns of "electron spin resonance" of the melanin present in the animal or vegetable substance considered.

Kerkut, Edwards and Munday have noted that there is a positive correlation between the concentration of free radicals and the degree of melanization and that an increase in free radicals could occur not only through the irradiation by short wavelengths (UV) but also by wavelengths of visible light as well as by the action of heating. Furthermore, it has been shown that oxidized melanin contains more free radicals than reduced melanin (Mason, Ingram and Allen) and that the degree of humidity of the pigment is directly proportional to the higher values of the patterns of "electron spin resonance" (Munday, Kerkut).

One fact appears from the experience of the abovementioned authors is that even light from an ordinary electric bulb, whose spectrum is well within the longer wavelength, can influence the free electrons of melanin just like the ultraviolet light even though in a lesser degree. The same authors have interpreted the effect of temperature on melanin as being produced by the molecular excitation which has probably increased the kinetic energy of the electrons with the breaking of bonds.

Melanin can therefore act as a semiconductor as well as an oxydo-reductive system. One important fact, to be explained below, is that this pigment is a very stable polymer and that the possibility of capturing or giving up electrons is related to melanin in all stages of maturation (indeed no free radicals were found during the phases of melanogenesis). Once the polymerization stage has been reached, it remains as a stable molecular construction that can only give up electrons or take on electrons in enzymatic oxydo-reductive processes and can also reach the degree of "maximum saturation" capturing electrons from the electromagnetic radiations striking it (Munday, Kerkut, Mason, Ingram, Allen) in the postulated conduction band of the quinoid chain. /841

It is therefore clear that the melanin plays an important metabolic role and that it can have an effect on the distribution of transfers of energy from all the endocellular material influencing the system or electron transfer according to specific and related plans.

The recently acquired findings on the submicroscopic structure as well as the enzymatic equipment of the basic "melanosome" appear to prove this assumption.

For example, insofar as concerns the activity of the dehydrogenases, Mian has reported that the latter are located within the melanocyte but to a degree relatively less than prevailing in the contiguous cells arranged along the germinative epidermal layer. The suggestion was made in these reports to note how the accumulation of pigment in one melanocytic unit could be connected to an increase in the dehydrogenasic activity within the same cellular unit.

The comparative estimate allowed finding in the complex that within the limits of the clear dentritic cell, the succino-dehydrogenasic and DPNH-diaphorasic activity was more intense in the elements with less pigment than in those which had a higher concentration of melanic accumulation.

The spacing out of the area of positivity was even clearer in the melanodendrocytes not appearing in the contiguous germinative elements and in the malpighian body. Such discoveries confirm the item of evidence concerning the singular antithesis of behavior between the localizations of dehydrogenasic activity, on one hand, and on the seats of melanic density and tyrosinasic activation, on the other hand.

The antithesis between dehydrogenasic activity in the general sense and the tyrosinasic activity in the special sense can be an expression of the phenomenon of competition between oxydo-reductive catalytic processes through which the introduction of one presupposes the interruption of the other.

The same endoatmic activity of such dehydrogenasic stations could hence function, on a theoretical basis, by the moderating momentum of the tyrosinasic reaction and consequently the rate of melanogenesis. The latter will be seen to automatically become intensified and connected to the braking control of the latter in the presence of inhibiting circumstances which would not form an obstacle to the endoatmic activity itself.

We have considered it absolutely necessary to set forth on a synthetic basis these recent events, first of all to report our experimental data in order to supply the elements indispensable to an interpretation of the findings which we have encountered in the cochlea and for possible parallelism with the number of phenomena relating to melanogenesis to the effect that the pigmented elements of this site can have with cutaneous sources or other seats. /842

PERSONAL CONTRIBUTION

The research was carried out partly on 40 petrous human bones (18 male and 22 female: from fetuses 6 months old and ranging to adults 82 years old). The specimens were taken not later than 25 hours after death, fix-d in Lilly's solution for 20 days. Decalcification in nitric acid was then carried out (5% aqueous solution, changed every 24 hours) which occasionally, especially for the advanced stage, included a permanent dwelling of the specimen in the solution for ca. 20 days. Immersions of 24 to 48 hours, in aluminum potassium sulfate after which washing in running water for 24 to 48 hours.

The method of mixed inclusion was used:

Alcohol	at	80°	=	48 hours
Alcohol I	at	95°	=	24 hours
Alcohol II	at	95°	=	24 hours

Absolute alcohol I, II, III } 12-24 hours in total

Methyl-benzoat-celloidin I = 48 hours
II = 48 hours
III until the specimen does not appear transparent

Benzol I = 1 hour; benzol II = 1 hour or 1½ hours, depending on size of specimens.

Paraffin I at 49° for 2 days, changed 1-2 times.

Paraffin 55° for 48 hours, changed once.

Inclusion in paraffin at 60°.

The included specimens are then microsectioned to 12-15 μ, given the difficulty of maintaining the sections whole and the topographic relationships of the membranous endocochlear microstructures.

Successively, in order not to produce an excessive blackening by the melanin (as is confirmed using stains of Masson, Gomori, Achucaro-Del Rio Hortega) which excessively masks the endocellular structure, we have used the 1% argentic impregnation, at ambient temperature for 24 hours, contrasting with carmalum for 5 min (method of Lignac: part one). /843

As test for differential destaining by other pigments, we used potassium permanganate, H_2O_2 (Hueck's test) for a number of sections while it was found not soluble in formaldehyde, alkalis, weak acids, alcohol, xylol, chloroform, benzol, ethylic ether, acetone and carbon sulfide.

HISTOLOGICAL FINDINGS

In a way limited to the goals proposed for this work, the histological sections have shown, in agreement with what was revealed by Beck in guinea pigs, a constant and considerably greater concentration of melanic pigment in the stria vascularis of the apical section, with respect to that of the basal section, without distinction between the two sexes. Even the age of the subjects had no effect on these relationships although there were substantial quantitative individual differences. In the fetuses it was not possible to make this comparative evaluation since melanocytes "were not shown". The melanocytes begin to assume the argentic tinting in babies 1-2 years old. The maximum concentration of pigment in phenotypically similar individuals with general pigmentation occurred around 60 years of age. From this age up to 82 years of age, corresponding to the most advanced age of our controlled individuals, we saw no modifications to any considerable extent in the content "of melanin" of the stria vascularis.

We have spoken specifically of melanin because the histochemical differential reactions performed by us have shown the involvement of this polymer in accordance with opinions set forth by other authors.

With a small magnification in the panoramic view of various sections, this diverse topographic distribution, under close examination, appears already appreciable but becomes even clearer with greater magnification (Figures 8 and 9) amounting to as much as 800-1000 X at which time it is possible to distinguish, inside the cell, the granular structure of the pigment which, for the density of the grains with lesser magnifications, gives the impression of being made up of amorphous clumps.

The examination which we carried out always amounted to comparing not only on a quantitative basis the melanin present in the various sections but to analyzing the distribution of the endo- and, if necessary, /846
the extracellular grains which were available for observation.

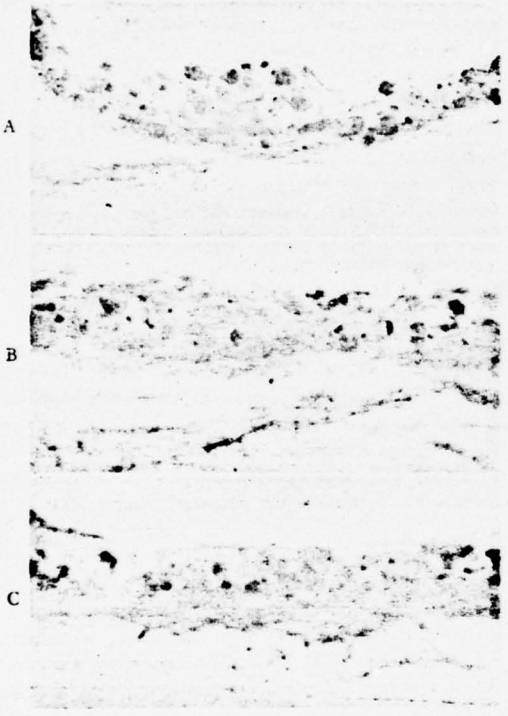

FIG. 8

Microfotografie di stria vascolare umana (donna: 62 anni) fissata dopo 20 ore, circa, dalla morte. Sezioni di 15 micron, colorate con impregnazione argentica (all'1%) in temperatura ambiente per 24 ore (secondo Lignac) e contrastate con carmallume (Ingrandimento x 800).

A - B - C - Microsezioni della stria vascolare in corrispondenza rispettivamente del giro apicale, intermedio e basale. È evidente la maggiore concentrazione di melanina nel giro apicale, rispetto al medio e al basale e così pure dei melanociti normali (di tipo epidermico), dei melanociti più piccoli (di tipo dermico) prevalentemente a disposizione perivasale, dei « sincizi di melanociti », dei « melanociti ipertrofici » e delle cellule epiteliali contenenti granuli di pigmento melanico.

Figure 8. Microphotographs of the human stria vascularis (female: 62 years old), fixed after ca. 20 hours following death. 15 micron sections, stained with a silver impregnation (at 1%) in ambient temperature for 24 hours (according to Lignac) and contrasted with carmalum (magnification 800 X).

A,B,C. Microsections of the stria vascularis in correspondence respectively with the apical, intermediate and basal section. Clearly seen is the greater concentration of melanin in the apical section with respect to the intermediate and basal sections. The melanocytes are almost all normal ones (of the epidermal type). There are smaller melanocytes (of the dermal type) predominantly in a perivasal arrangement. There are "syncytia of melanocytes", "hypertrophic melanocytes" and epithelial cells containing grains of melanic pigment.

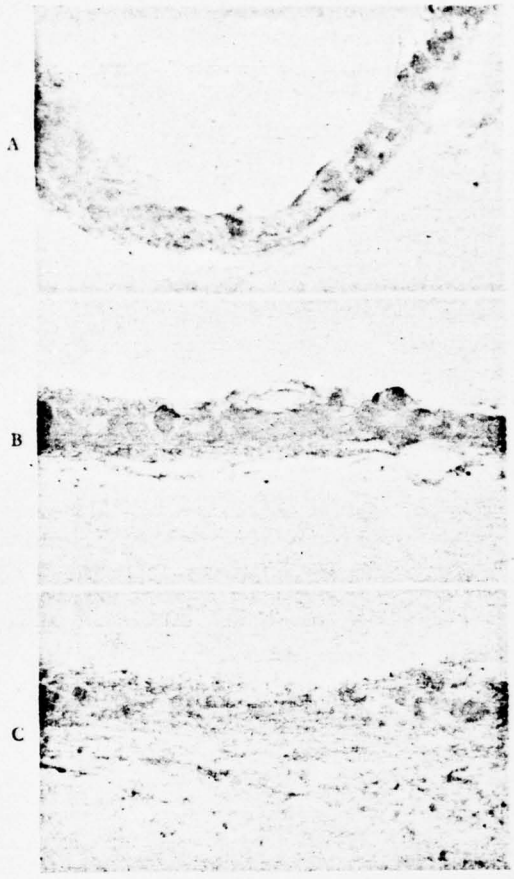

FIG. 9

Microfotografie di stria vascolare umana (uomo: 60 anni) fissata dopo 20 ore, circa, dalla morte (impregnazione argentica secondo Lignac).

A - giro apicale. B - giro intermedio. C - giro basale. (Ingrandimento x 800)

Anche in queste microsezioni è evidente la maggiore concentrazione di melanina nel giro apicale e nel medio rispetto al basale e così pure degli elementi pigmentati. In B) è visibile un grosso capillare sezionato, sporgente nel lume cocleare, ed una voluminosa cellula epiteliale vescicolare in fase secretoria, contenente granuli di melanina di completa maturazione.

Figure 9. Microphotographs of human stria vascularis (male: 60 years of age) fixed after ca. 20 hours following death (silver impregnation according to Lignac). /845

A: Apical section
B: Intermediate section
C: Basal section
(Magnification 800 X)

Also seen in these microsections is the greater concentration of melanin in the apical section as well as in the intermediate section with respect to the basal one. These are almost all pigmented elements. In B can be seen a large sectioned capillary projecting into the cochleal cavity as well as a voluminous vescicular epithelial cell in secreting phase, containing completely mature melanin grains.

One common phenomenon, noted in all preparations examined, is the loss of one part of the grains owing to their departure from the cell containing them related to the very characteristics of the grains which at the stage of maturity are found in the melanocyte free in the cytoplasm and limited in their movements only by the density of the medium.

This still does not prevent providing a quantitative evaluation and a topographical differential of this pigment in the stria vascularis since it is a phenomenon which occurs at the level of all the sections.

Hence, with these premises and given the repetitiveness of findings, we are able to establish that the modest verification of the phenomenon is not such to change the comparative evaluation carried out by us.

FIG. 10 - 1-2-3

Melanociti ipertrofici (o giganti) in mezzo a melanociti normali. Sezione di cute sottoposta precedentemente a lunga esposizione di luce solare e trattata con Dopa (Ingrand. x 279).

(*da:* SNELL)

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Figure 10. 1,2,3. Hypertrophic (or giant) melanocytes in the middle with normal melanocytes. Section of skin subjected previously to long exposure of solar light and treated with dopa (magnification 279 X). /847

(By: Snell)

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Another datum which we should like to emphasize is that the postmortem degradations encountered in the tissues and the phaneroses of the endocellular pigment, always done by postmortem chemical process, if we can consider one fixed parametric index, is either because an almost equal length of time has taken place from the death of the subject to the fixation of the specimen or because it involves modifications which are confirmed in the whole cochlea.

The following data were revealed from observation in immersion of the preparations: In the microsections of the stria, at each cochlear level, there appeared formations having quite diverse volumes and shapes, containing silver-stained grains and delimited by membranes. However, it is possible to see groups of linear segments of these grains almost appearing to be located with a cytoplasmatic pellicle like a sheath in the interstice of adjacent cells or superposed on small vessels.

These patterns are not easy to interpret. Indeed, the pigmented

macroelements in which it is possible to occasionally make out one or more voluminous nuclei masked by grains of endocytoplasmatic melanin showing various degrees of silver affinity (chestnut color gradations) appeared in various forms from element to element. Having made the comparisons with those skins under conditions of normality and actinic stimulation (Figures 10 and 11), we believe we can recognize in these cells melanocytes in various phases of maturation and functional activity with characteristics which put them close to epidermal melanocytes. While we consider that the other formations with a small section, anucleate, circumscribed by a limiting membrane, with a cytoplasmatic halo and small groups of grains may be more or less oblique transverse sections of dendrites of melanocytes either located in proximity of those which can be seen in the sections or appearing to be distant, it appears probable that they could be- /848
long to dislocated elements with the cellular body in other levels.

The semidiagrammatic reproduction of Leimgruber (Figure 12), the aspect of the epidermal melanocytes (Figures 10 and 11) and of some of those appearing in the intravital microphotographs of the lateral membranous semicircular canal of normochromic guinea pigs (Figure 13) and of the perilymphatic membrane of the internal ear of rana esculenta (Figure 14B) (Cherubino. Bonaccorsi, Galioto) justify this interpretation by the fine arborescent prolongations shown by these cells in the expansion phase.

FIG. 11

Sezioni di cute di 7 micron: melanocita in mitosi. Si nota la presenza di granuli di premelanina. Immersione in olio x 1125.

(da: MITCHELL)

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Figure 11. 7 micron sections of skin: melanocyte in mitoses. There may be seen the presence of premelanin grains. Oil immersion. 1125 X.

(By: Mitchell)

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In addition to these formations, there can be seen in the sections cells with lesser dimensions and nonuniform shape arranged linearly and whose grains show the same tinted property as those of the more voluminous elements previously described and which we then interpret as melanocytes smaller in size and in various activity phases, similar to the

melanocytes of the "derma": Indeed, these are more frequently than the others in a perivasal arrangement in "pericytic position".

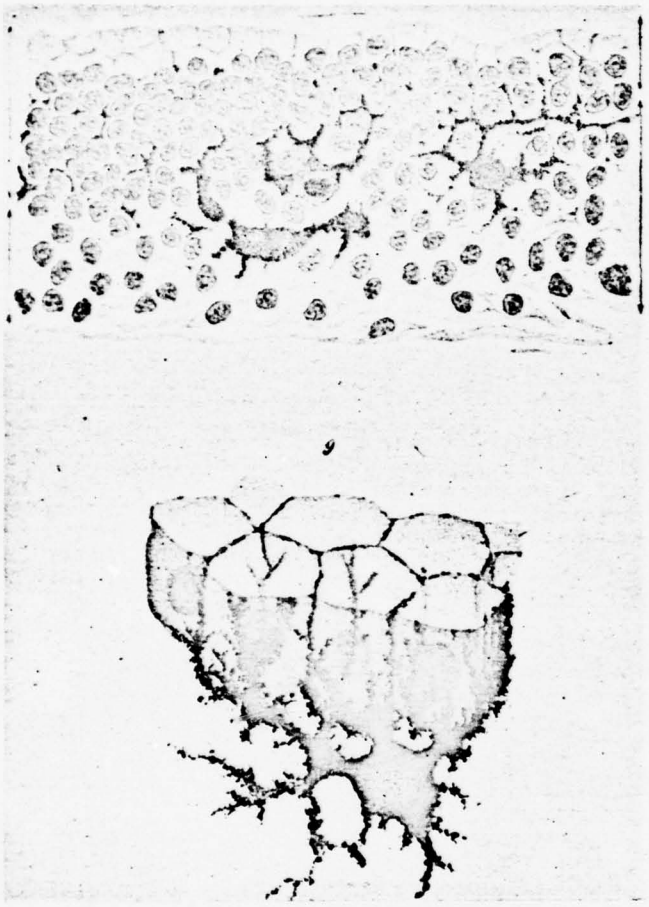

Figure 12. 5.2 cm guinea pig embryo: Lateral wall of the cochlear canal. Behavior of pigmented cells with respect to the epithelium, in semidiagrammatic reproduction. /849

(By: Leimgruber)

FIG. 12

Embrione di cavia di 5,2 cm.: parete laterale del canale cocleare. Comportamento delle cellule pigmentate rispetto agli epiteli, in riproduzione semischematica.

(*da:* LEIMGRUBER)

A significant detail resulting from observation of microsections of /851 the stria vascularis is that, in a number of cases, at the level of the apex, there are groups of melanocytes apparently in syncytial unions and appearing to constitute voluminous pigmented polynucleate cells (Figure 14A,C). Nevertheless, independently from the volume of the cells, the stain characteristics of the grains are the same for each section as is clear from examining the macro- and microelements (nucleate and anucleate) of the two extreme sections: the apical and the basal (Figures 8 and 9).

Indeed, in this latter one, all of the pigmented cells, furthermore scanty, assume a slight yellowish tinge, so much so as to hardly be able to be differentiated within the context of the tissue and naturally their slight affinity for silver is an index of the incomplete maturity of the melanin grains.

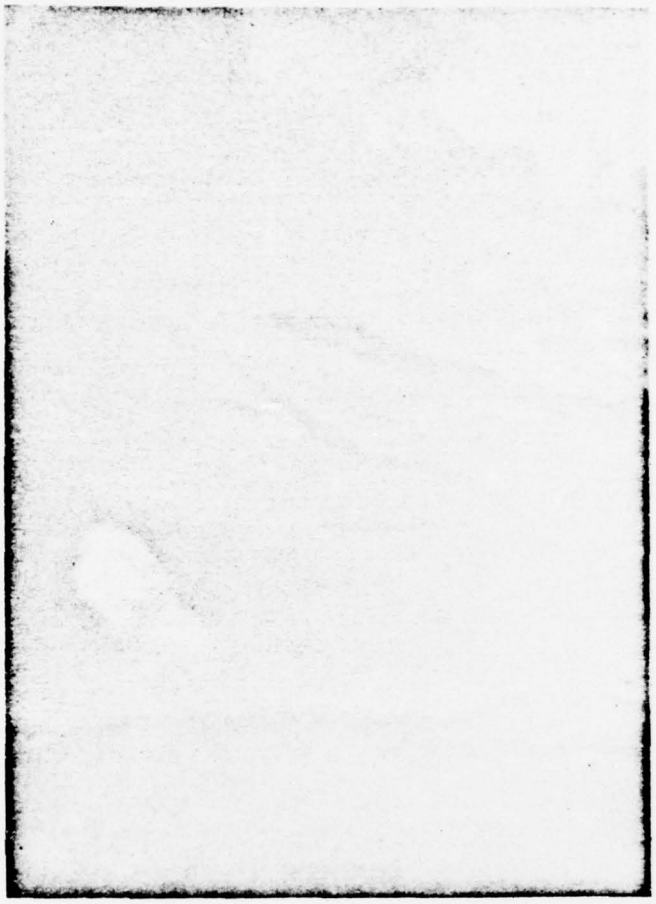

FIG. 13

Microfotografia intravitale del canale semicircolare laterale membranoso di cavia normocromica eseguita senza alcuna colorazione: si vedono chiaramente melanociti in varia fase di attività (a palla, aracniformi), di cui alcuni disposti intorno ad una arteriola in corrispondenza di una biforcazione.

(da: CHERUBINO, BONACCORSI, GALIOTO)

Figure 13. Intravital microphotograph of the lateral membranous semicircular canal of the normochromic guinea pig performed without any tinting: There can be clearly seen melanocytes in various phases of activity (ball-shaped, arachniform), some of which arranged surrounding a small arteriole connected with a bifurcation. /850

(By: Cherubino, Bonaccorsi, Galioto)

Furthermore, progressing in observation from apex to base, we find a considerable reduction in all the melanocytes (macro, micro, syncytes) or at least a reduction in their stain property.

One other datum which resulted from the histological preparations is the presence of melanin grains also in the epithelial cells and above all in those in the secretive phase in the high cochlear section. In these cells, the pigment when it is present is made up of uniform intense dark grains just like those contained in the melanocytes with a greater enzymatic activity and like those which commonly are found in the apical and medium-level sections of the stria vascularis with noteworthy individual differences as furthermore we have remarked for all the elements of the melanogenetic system whereas it is practically absent in the basal section.

CONSIDERATIONS

It is beyond the scope of our research to make any attempt to interpret the terms and conditions for removing the melanin grains which are produced by the melanocytes in the stria vascularis. Still, the encounter of these grains also in the epithelial cells suggests that in the stria vascularis there can occur the same phenomena as found in the epidermis.

Cruickshank and Harcourt ascertained using "time lapse" photography on suspensions of epidermis from humans and guinea pigs "in vitro" in special chambers, how the transition of pigment to "keratinocytes" in culture, principally occurred in two modes: /853

> In the first (Figures 15 and 16), the terminal part of the dendrite is dilated, the cytoplasm becomes more fluid, the cellular membrane in the keratinocyte is lifted as if the two formations were reciprocally attracted. Coming into contact, the extremity of the dendrite is folded allowing escape of groups of grains which penetrate in this way into the cell. At no time was there observed the passage of a single grain. It is also seen that the melanocyte can give more than one epithelial cell at the same time and, on the other hand, that one epithelial cell can receive grains for more than one melanocyte;
>
> In the second process, the dendrite is located along the surface of the epithelial cell, the grains move toward the terminal part of the dendrite which is detached forming a group of grains. Similarly, the latter show rapid undulating movements and phagocytes come from the epithelial cell as a group still surrounded by a pellicle of cytoplasm. The remaining dendrite quickly withdraws. /854

Hence, there is to some extent direct intracellular emission as well as a process of phagocytes.

According to quite recent investigations (Wassermann), beyond the melanophores present in the skin and capable of contraction and expansion under hormonal stimulus (alpha-MSH and beta-MSH) the leukocytes (lymphocytes, granulocytes and monocytes) will also enter into the process of /855 extramelanocyte migration of grains of melanin and will be encountered in the peripheral blood in reptiles, amphibians and even in man, under normal conditions, in the pigmented races (Backer, Wassermann) especially on the occasions of septic cutaneous inflammation (Wassermann) or traumatisms of melanotic tumors (Ishjzaki) in the white race as well.

However, it appears (Masson, Mitchell) that the melanocytes of the epidermis can transfer grains with pseudopods through the basal membrane as well as macrophages and melanophores of the skin, distributing grains, hence in two directions.

Flows of dark melanin grains, also without dopa staining, have been seen by Mitchell in the dendritic connections of melanocytes with vector cells. When the latter are full with pigment, they appear to go more deeply and even to assume perivasal positions.

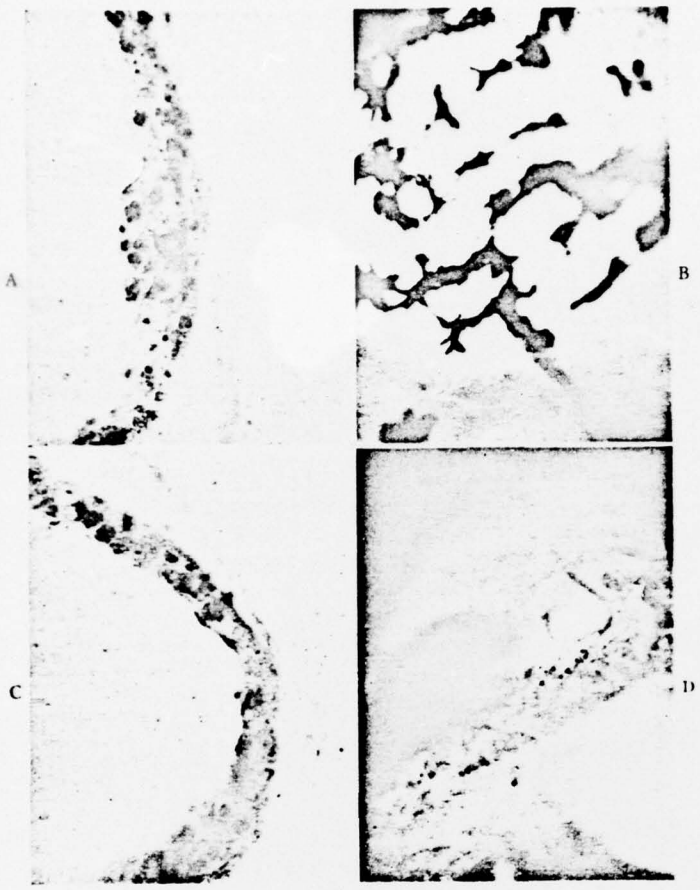

FIG. 14

Visione d'insieme di quadri istologici ed intravitali:

A - C - Microsezioni istologiche di stria vascolare umana (le stesse della fig. 8 e 9) del giro apicale, trattate con impregnazione argentica secondo Lignac.

B - D - Microfotografie intravitali rispettivamente della membrana perilinfatica dell'orecchio interno di rana esculenta e della stria vascolare (terza spira) di cavia normocromica, così come appaiono alla osservazione diretta, senza alcuna colorazione.

(*da*: CHERUBINO, BONACCORSI, GALIOTO)

Dal confronto dei quadri istologici con gli intravitali, controllando anche la ricostruzione semischematica (Leingruber) e i rapporti tra melanociti ed epitelio della parete laterale del condotto menibranoso cocleare embrionale della cavia (fig. 12) si può intuire come siano, « in vivo », le unioni sinciziali (B-D) tra melanociti (da due a più elementi). Questa particolare « convivenza cellulare » è meglio evidente nella microfotografia della membrana perilinfatica della rana (B).

Figure 14. General view of histological and intravital patterns: /852

A,C. Histological microsections of human stria vascularis (the same as Figures 8 and 9) of the apical tract, treated with silver impregnation according to Lignac.

B,C. Intravital microphotographs respectively of the perilymphatic membrane of the internal ear of rana esculenta and the stria vascularis (third section) of a normochromic guinea pig just as they appeared to direct observation without any staining.

(By: Cherubino, Bonaccorsi, Galioto)

From the comparison of the histological patterns with the intravital patterns, also checking the semidiagrammatic reconstruction (Leimgruber) and the relationships between melanocytes and epithelium of the lateral walls of the cochlear embryono membrane duct of the guinea pig (Figure 12), it is possible to perceive how the syncytial unions (B-D) could be "in vivo" between melanocytes (of two or more elements). This special "cellular cohabitation" is clearer in the microphotograph of the perilymphatic membrane of the frog (B).

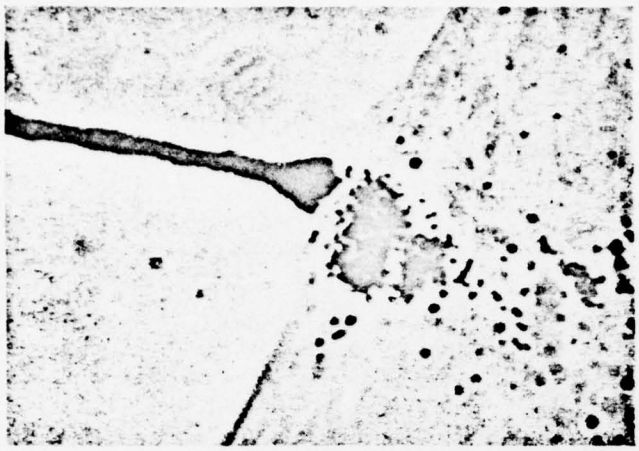

FIG. 15

Cultura in vitro di sospensioni di cellule epidermiche di cavia normocromica (13 giorni). È illustrato chiaramente il passaggio di un gruppo di granuli di melanina da un dendrite in una cellula epiteliale.

(*da:* CRUICKSHANK, HARCOURT)

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Figure 15. Culture in vitro of suspensions of epidermal cells of a normochromic guinea pig (13 days). This provides clear illustration of passage of a group of grains of melanin from a dendrite into an epithelial cell.

(By: Cruickshank, Harcourt)

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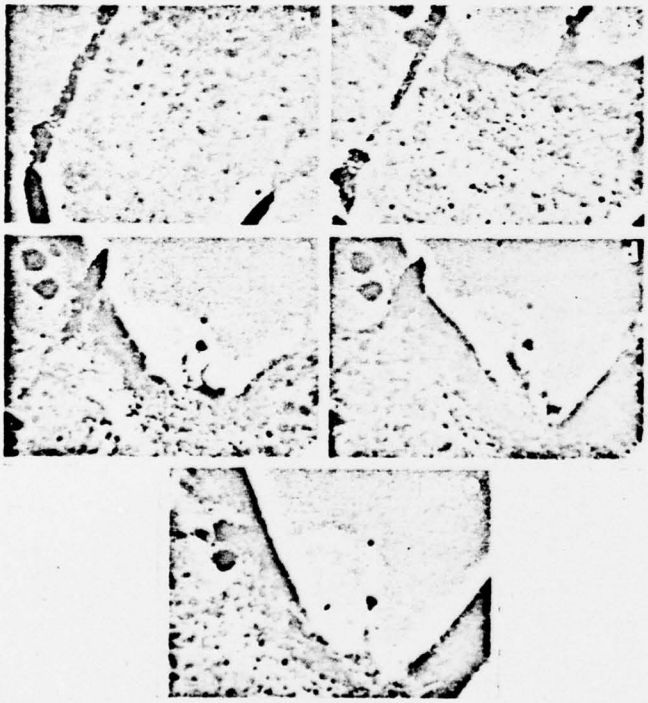

FIG. 16

Cultura in vitro di sospensioni di cellule epidermiche di cavia normocromica (35 giorni): *a*) il dendrite di un melanocita è situato attraverso la cellula epiteliale ed il citoplasma incomincia a diventare più fluido (0 minuti), *b*) il dendrite si spezza (29 minuti), *c*) il nucleo, nella cellula epidermica appare in alto a sinistra (49 minuti), *d*) sta fagocitando un gruppo di granuli di melanina (62 minuti), *e*) (76 minuti).

(*da:* CRUICKSHANK, HARCOURT)

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Figure 16. Culture in vitro of suspensions of epidermal cells of a normochromic guinea pig (35 days).

a. The dendrite of a melanocyte is located through the epithelial cell and the cytoplasm is beginning to become more fluid (zero minute),

b. The dendrite breaks up (29 minutes),

c. The nucleus in the epidermal cell appears top left (49 minutes),

d. A phagocyte process with a group of melanin grains (62 minutes),

e. (76 minutes).

(By: Cruickshank, Harcourt)

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It appears, according to Birbeck, that the melanophores of the skin can synthesize melanin grains as epidermal melanocytes, but will not be differentiated by being mobile and by holding back the pigment inside their own cytoplasm becoming effector cells.

The study with the electron microscope of the ultrastructure of the pigmented formations of the stria vascularis and other seats of the internal ear can help understanding and interpreting the many morphological and functional problems of these cells which, owing to the discriminative power of the optical microscope, given the masking of the grains, can only be made hypothetically.

The magnitude of this problem becomes clear from our preceding investigations (Cherubino, Bonaccorsi, Galioto) on a number of morphological and functional aspects of the elements of the melanogenetic system carried out on the rana esculenta and on normochromic and albino guinea pigs.

Having made these premises (to the extent that it is not possible to discuss melanin, hence the melanin grain, without considering the cell producing it and carrying it as well as the terms and conditions of distribution), the fundamental question arising from our investigation is the one related to the finding of a large quantity of melanin and hence of melanocytes, detectable ones, at the apex with respect to the base of the cochlea, not only in guinea pigs, as has already been shown by Beck, but also in man. This suggests, given the varying position of the human cochlea, deeply situated in the cranial vault, protected by the thickness of compact bone of the pars petrosa, compared with that of the guinea pig, projected freely into the air cavity of the bulla and hence much more superficial compared with the integuments, that such distribution of pigment in the cochlear canal is not a function of the influence of "light" but is connected with the morphology and structure of this type of acoustic apparatus, arranged in spiral form and that overall it is a conic section.

The consideration whereby, according to the traditional concept, the melanin should provide protection from ultraviolet rays, or according to somewhat more recent concepts even by now out of date, from radiations in general, did not find justification in this organ. Indeed, if in the guinea pig through the special seat of the cochlea which is found with the apex more exposed in the cavity of the bulla, covered with a much thinner periotic capsule than at the base, it would be possible to assume the capability of easier exposure to radiations and hence by this to justify the greater development, in the upper tracts, of the melanogenetic system, that is excluding the human cochlea, shifted in another seat, and that it will never be able to receive more light in special incidence through the tympanum and the fenestra cochleae with reversal, in such case, of the pigment distribution. /856

The unequal location of melanin, hence, excludes this pigment from having a protective role involving radiation. In such a case, there would be a uniform distribution in the whole cochlear canal.

The assumption that in an ectodermal organ, which in a certain phase of its ontogenetic and phylogenetic development is found open to the outside,

justifies the presence of melanic pigment (Catalano, Madonia) could be an attempt for an interpretation leaving unsolved problems. First among these would be that involving the exact location in connection with the microvasal plexi of production and of reabsorption of endolymph, whether in the anterior or posterior labyrinth. In the second place, insofar as regards the acoustic labyrinth, it leaves without response the differing pigmentary concentration clearly greater at the apex, even in man, as our investigations have verified and which we cannot consider to be accidental.

There are admissible individual differences connected with the genotypical characteristics. There is, however, a constant topographical pattern of the pigmentary cochlear planimetry owing to the rationality of ultimate biological laws which have allowed evolution of the animal and vegetable species and still exert on our generations their bio-histioformative power. Hence, there should be a biological justification. After having evaluated all of these assumptions, we have formed our opinion on these problems in a manner consistent with biophysical laws.

The function of Corti's organ is closely correlated to the constancy of a number of biochemical and physical characteristics of the endolymph and perilymph which in turn operate in the complex phenomenon of selective and secretory permeability basically integrated into the two-term phrase: "Capillary endothelium" - "epithelial cells" of the stria vascularis. /857
It is for this reason implicit that any kind of minimum change in the physiological equilibrium owing to cytological disorder at this level, especially in the capillary walls which are greatly exposed to hemodynamic stresses, delays production of endolymph with constant chemical-physical changes resulting in harm to the sensorial cells.

Now, in an organ with a conical section like the cochlea, with undeformable walls, there can occur a number of phenomena connected with the propagation of the vibrational wave, that is the instantaneous molecular attrition (the acoustic wave having a sinusoidal character) and through all frequencies and through the greater intensity at the point not at the base of the cochlear canal.

Indeed, considering the labyrinthine liquids to be incompressible and the walls of the cochlea to be rigid, the following relationship between velocity and area of the sections along the axis of the acoustic labyrinth is as follows:

$$S_1 V_1 = S_2 V_2$$

in which S_1 is the larger section of the base of the cochlea, V_1 the instantaneous velocity of the vibrational movement at this level, S_2 the minor section of the cochlear apex and V_2 the instantaneous velocity of the relative vibrational movement, and it follows that "the velocities are inversely proportional to the sections".

Hence, during the vibration, the velocity of the liquid in the slender area of the canal is greater than the velocity in the area in proximity to the stirrup bone.

On the other hand, it is noted that "the force of attraction" exerted by a layer of liquid on the walls of a duct, owing to the viscosity effect is given by:

$$F = \eta \frac{V\ A}{h}$$

in which V is the velocity, A the surface of the layer, h its distance from the wall, and η the viscosity coefficient of the liquid. It results from this that, within the slender areas of the cochlea, there are attractive forces of the liquid on the walls that are greater than those exerted on the walls of the area with a greater section.

Such diversity of "friction" is reflected in a different stress for wear of the walls and consequently in a "different intensity of production of heat by friction".

For this reason, it is possible to physically explain how the stria vascularis, in correspondence with the apex, undergoes the most mechanical stresses and hence, potentially, the greater vascular-epithelial injuries, which with equal frequency result proportional to the intensity of the sound.

Hence, there may be given according to this postulate a phylogenetic /859
justification to this typical location of melanin, melanocytes or even better the melanin grain. This is a biologically active unity now considered as the substrate of the oxydo-reductive systems and the latter is functionally considered a biological semiconductor, reactive noncontinuous source of electrons in every case of cellular pathergy of a mechanical-vibratory nature through the property inherent in the polymeric molecule of the melanin itself to supply free radicals by breaking the bonds of union following upon all instantaneous rises in temperature which are verified at this level as has been experimentally demonstrated with measurements of the "electron spin resonance" spectrum in other seats (Munday, Kerkut).

Furthermore, the stimulation and local melanogenetic activation explains the presence of "hypertrophic melanocytes" and "syncytes of melanocytes" which are sometimes encountered in the apical tract, in the sections of the human cochlea examined which found a relationship in the biomicroscopy of the same seat in normochromic guinea pigs (Figure 17) with morphological analogy as occurs in the "epidermal melanocytes" exposed to intense actinic stimulations (radiation in the ultraviolet, X, α and γ regions) (Quevedo, Grahn, Szabo, Mitchell).

It is true also that the whole cochlear melanogenetic system can feel the effects (as in other locations) of hormonal activation (intermedin, Figure 18): Atwell, Hogben, Winton, Frieden, Bozer, Lerner, Shizme, Bunding, Karkun, Mukerji, Chavin, Lerner, McGuire, Snell; estrogen-progesterone: Snell, Bischitz, Snell; and the neurovegetative control (Bathman).

From this it can be considered that the circulation is not at all alien to pigmentation. Indeed, the afflux of chromogenes and oligosynergic coeffectors (branches, etc.) comes into the melanogeneses as an

irrigating agency. The same stimuli considered adequate to promote the synthesis of the pigment (actinic radiations as well as ultraviolet and infrared radiation, cosmic rays, heat) are at the same time adequate to cause, with an angiotropic effect, a vessel response with gradation of intensity passing from infraliminal effects to an obviously pathogenic reaction. From this, the importance is clear whereby it is possible to have even phylogenetically a correlation between vascular disorder owing to supraliminal intensity of sounds and the chief locations of melanocytes at the apex of the cochlea by contributing with their granular enzymatic set "reserve energy sources" through local biochemism.

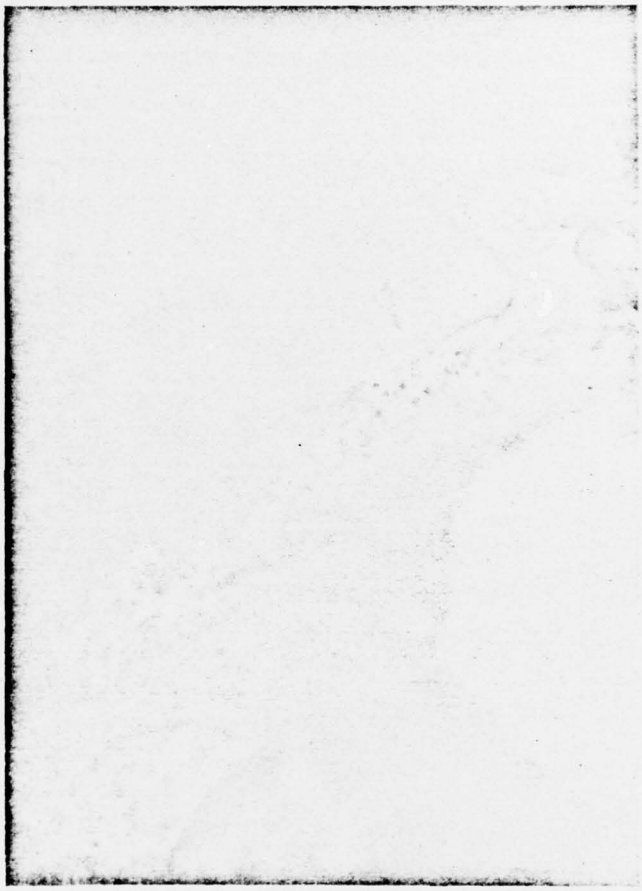

FIG. 17

Stria vascolare di cavia normocromica (breccia ossea in corrispondenza del terzo giro). (Ingrandimento della figura 1-A, per meglio evidenziare la morfologia e topografia degli elementi del sistema melanogenetico).

(da: CHERUBINO, BONACCORSI, GALIOTO)

Per gentile concessione della Williams & Wilkins Co. di Baltimora.

Figure 17. Stria vascularis of a normochromic guinea pig (bone opening corresponding to the third tract). (Magnification of figure 1A in order to better show the morphology and topography of the elements of the melanogenetic system.)

(By: Cherubino, Bonaccorsi, Galioto)

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/858

Indeed, in the cochlea and more particularly in the stria vascularis in Corti's organ in the structure of the external spiral tract where the metabolic processes are more active, there is a larger enzymatic content.

In a comparative analytical examination, the various topographic distributions of the dehydrogenases affect the stria vascularis and the spiral connection. Thus, the dehydrogenases (succinic and the DPN diaphorases) are encountered in considerably larger concentrations at the base than at the apex (Yoshikawa, Morimoto, Koide, Hando, Yosikawa) (Figure 19) whereas the cytochromo-oxydase has a uniform distribution in the whole section (Yoshikawa, Morimoto, Conti, Borgo). /860

Functional tests also carried out by the authors indicated above have shown that prolonged acoustic stimulation reduces the quantity of dehydrogenases present in the stria vascularis especially in the apical tract, in the center as well as the lower one according to whether the frequencies used are low or high respectively whereas an overall decrease occurs for the cytochromo-oxydase independently from the frequency impressed on the stresses.

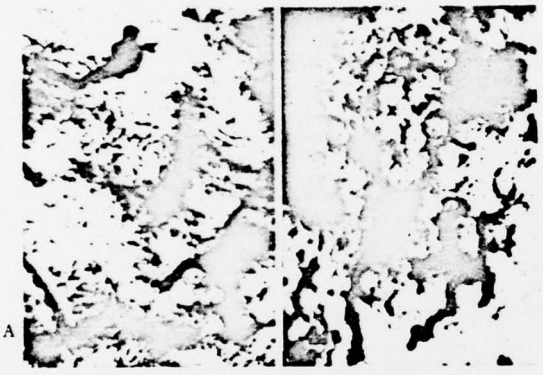

FIG. 18

Microfotografie della pelle della parete anteriore dell'addome di cavia normocromica:

A - aspetto dei melanociti prima del trattamento;
B - dopo trattamento con alfa-M.S.H.: il numero dei melanociti appare aumentato ed i complessi processi dendritici sono ricolmi di melanina (trattamento con Dopa - Ingr. x 885).

(*da*: SNELL)

Figure 18. Microphotographs of the skin of the anterior wall of the abdomen of a normochromic guinea pig:

A. View of melanocytes before treatment.

B. After treatment with alpha-MSH: the number of melanocytes appears increased and complex dendritic processes are filled with melanin (treatment with dopa -- magnification 885 X).

(By: Snell)

However, it is found that in each chain of enzymatic systems the functioning depends on the fact that the product of the fermentation action makes a specific substrate of the following enzyme. The cellular endoatmia, that is the transport of electrons without molecular oxygen, is essentially accomplished through the chain of cytochromes present in the mitochondria. /862

A respiratory chain such as this can be indicated diagrammatically as follows:

substrate - dehydrogenase - cytochrome - cytochromo - oxydase - O_2

from which the importance is clear of the reserve of dehydrogenase as a source for immediate utilization of energy for Corti's organ and for the

stria vascularis in the segments of the cochlea corresponding to the stimulization frequency.

From the comparative observation of the topographic distribution of the dehydrogenase, it is striking how the presence of the melanin in the stria vascularis is inversely proportional to the concentration of these respiratory enzymes. Such a correlation does not appear accidental to us because it corresponds to what was encountered by Mian in the epidermis and dermis where the same relationship exists.

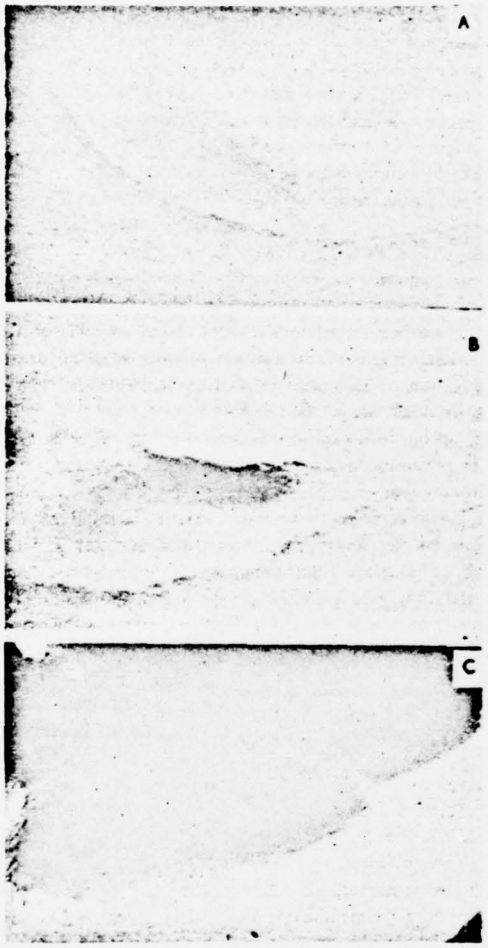

FIG. 19

Distribuzione della succinodeidrogenasi nella stria vascolare a livello del giro apicale (A) medio (B) e basale (C) di cavia (colorazione istochimica). È evidente la disposizione inversa, di quella della melanina, dall'apice alla base della coclea. La deidrogenasi è colorata in nero: risalta la sua massima concentrazione nel giro basale.

Figure 19. Distribution of the succinic dehydrogenase in the stria vascularis at the level of the apical (A) medium (B) and basal (C) tract of guinea pigs (histochemical staining). The reverse arrangement is clear on the part of the melanin from the apex to the base of the cochlea. The dehydrogenase is colored in black and results from its maximum concentration in the basal tract.

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Indeed, even in the cochlea, at the base where the dehydrogenase is prominent, we find that the melanin is scarce whereas the reverse is true at the apex.

The interpretation given to the "system" in the skin can thus be transferred to the cochlear level. It is probable that it involves enzymes with a balanced activity from which the dehydrogenase, by its very chemical structure, would be for immediate use whereas the melanin, as a polymer with an elevated stability, would take place as an auxiliary enzyme releasing electrons progressively with the reduction of the inhibiting preponderance of the former during its utilization.

According to this concept, melanin has a reserve enzymatic activity and explains its protective task for the neural epithelium at the cochlear level, thereby integrating the local oxydo-reductive processes.

According to this concept, melanin probably has an enzymatic reserve activity which would explain its protective task on the neuroepithelium at the cochlear level, to some extent integrating local oxydo-reductive processes deferring the phenomena of cellular hypoxia at the strial level which is expressed by the changed capillary permeability retarding the acoustic fatigue and later protecting the neuroepithelium from injuries caused by the acoustic trauma. Owing to this, the basal section, being the one possessing the least pigment, is found to be the most vulnerable.

Hence, theoretically, the individuals who, owing to genotypical make-up show less pigmentation of the stria vascularis, other parameters being equal, are probably more seriously damaged and on an earlier basis by intense and prolonged exposure to noise and in which there will have to be "that hypothetical constitutional factor" which determines the great difference in individual sensitivity for this type of cochlear injury.

Furthermore, the possibility should not be excluded of deep penetration by infrared radiation and partially cosmic radiation of the melanin owing to the high photoabsorptivity related to its molecular structure. /863
This does not explain in the internal ear the protective function commonly postulated with respect to radiation, rather it represents a substrate of accumulation of gradient energy passing through it but with the saturation of the reticulum by the magnetic molecular resonance which is reflected in modifications of the extranuclear structure of the molecules and atoms or in modifications of energy of rotation or vibration of the same molecules. The energy used in various forms of conversion (by production of free radicals or exchange of cations) appears to be a function of the progressive reduction of the dehydrogenasic activity and of the molecular stresses mechanical-vibrational in nature present in the cochlea.

According to this view of the problem, hence, the melanin and more analytically the melanin grain, can be considered the "energy resource" of the cochlea not absolutely necessary for its sensory function and for the life of its cells. However, in emergency conditions owing to excessive acoustic stimulations, it could enter into the chain of respiratory endocellular oxydo-reductions avoiding the danger or slowing down the effects of temporary or irreversible cochlear damage.

From this hypothetical concept, which requires experimental confirmation, results the "prophylactic" importance for preventive medicine of being able to recognize in the mass of individuals a test for evaluating the "concentration" of melanic pigment in the stria vascularis which could

be related to another easily observable phenotypical surface constant such as the expression of pleiotropism of a single gene.

Furthermore, as we have reported, it is understandable why researchers who have dealt with the argument have not found modifications in the histochemical reactions owing to melanin at the strial level following various stimuli to the extent that, in the latter, with complete maturity of the grain, there basically occur only intramolecular electronic mutations "of reticulum" as in semiconductors not demonstrable with chemical reactions but which impose recourse to electronic physics for their appraisal.

SUMMARY

Comparative research on the distribution of the melanin of the stria vascularis in the different sections of human cochlea. Functional interpretation regarding the structure of the melanin.

The author, on the basis of histological findings (40 petrous bones starting from the age of 6 months to 82 years), shows in the human "stria vascularis" that the same pigmentary structure can be found as in guinea pigs. In fact, the concentration of melanin is a major quantity in the apical tract of the cochlea and it decreases quantitatively in the lower tracts.

The research has a special significance because of the recent findings regarding presence of free radicals of melanin. Such pigment could be considered as a factor in the oxydo-reductive system of the labyrinth and could also have the function of a biological semiconductor.

As a matter of fact, the author states that the apical tract of the cochlea is subjected to most of the mechanical influences and, for this reason, the vessels and epithelium have the potential possibilities for more frequent stress.

The author also observes, as in the cochlea, that there is an inverse quantitative relation between melanin and dehydrogenasis.

The author states that the "melanin grain" can be considered the source of energy of the cochlea and (although not indispensable to the vital function of the cochlea itself), it participates in emergency requests to the respiratory oxydo-reductive function of the cells avoiding or reducing a possible temporary or permanent cochlear damage.

BIBLIOGRAPHY

/865

ADAMS M., BLOIS M. S., SANDS R. H., 1958. — J Chem. Phys., 28, 774. Citato da MUNDAY e KERKUT.

ALEXANDER G., 1901. — *Das Labyrinth Pigment des Menschen und der höhere Säugetiere*. Arch. Mikr. Anat., 58, 134.

ATWELL W. J., 1919. — *On the nature of the pigmentation changes following hypophysectomy in the frog larva*. Science, 49, 48.

BAKER W. DE C., 1964. — *Melanin deposits in the lymph glands of Africans in Kenya*. E. Afr. Med. J., 41, 15.

BECK C., 1961. — *Das Pigment der Stria Vascularis*. Arch. fur Ohren, Nasen, und Kehlkopfheil, 179, 51.

BIRBECK M. S. C., 1962. — *Electron microscopy of melanocytes*. Brit. Med. Bull., 18, 220.

BISCHITZ P. G., SNELL R. S., 1960. — *A study of the effect of ovariectomy, oestrogen and progesterone on the melanocytes and melanin in the skin of the female guinea-pig*. J. Endocr., 20, 312.

BONACCORSI P., 1963. — *L'albinismo e le sue correlazioni in Otorinolaringologia: ricerche funzionali ed ipotesi interpretative sull'attività melanogenetica*. Boll. Mal. O.G.N., 81, 408.

/866

BONACCORSI P., 1963. — *Comportamento delle barriere emolabirintica, liquorale ed oftalmica nell'Albinismo*. Ann. Lar. Otol. Rinol. Faringol., 62, 432.

BORGHESAN E., 1962. — *The reticular zone of the planum semilunatum*. Acta Otolaryng. (Stock.), 54, 27.

—, 1964. — *Nature of a pigmented substance in the labyrinth*. Acta Otolaryngol. (Stock.), 57, 288.

CASSIDY H. G., 1949. — J. Amer. Chem. Soc., 71, 402. Citato da MUNDAY e KERKUT.

CATALANO G. B., MADONIA T., 1962. — *Distribution et aspects du pigment dans les structures labyrinthiques*. Rev. de Laryng., 83, 1141.

CATALANO G. B., MADONIA T., DE MARIA R., 1963. — *Considerazioni morfofunzionali sulle cellule pigmentate del planum semilunatum*. La Clinica Otorinolaring., 15, 1.

CATALANO G. B., MADONIA T., 1964. — *Le cellule pigmentate dell'orecchio interno*. La Clinica Otorinolaring., 16, 3.

CHERUBINO M., GALIOTO G. B., SAMBUCO G., BONACCORSI P., MIRA E., DAL NEGRO F., 1964. — *L'emoangiodinamica cocleare*. Rel. XXII Raduno Otorinolaring. Alta Italia, Pavia 8 dicembre.

CHERUBINO M., GALIOTO G. B., BONACCORSI P., SAMBUCO G., 1965. — *La microcircolazione intravitale del labirinto acustico e vestibolare*. Rel. II Congresso Microcircol. Como, ottobre.

CHERUBINO M., BONACCORSI P., GALIOTO G. B., 1965. — *Gli elementi pigmentati del sistema melanogenetico nella microangiologia dell'orecchio interno*. Rel. II Congresso Microcircol. Como, ottobre.

—. *Considerazioni sulla biomicroscopia delle cellule pigmentate del sistema melanogenetico del labirinto acustico e vestibolare*. Boll. Soc. Med. Chirurgica, Pavia.

—. *Sul comportamento dei melanociti nell'orecchio interno in presenza di stimoli di natura diversa*. Boll. Soc. Med. Chirurgica, Pavia.

COMMONER B., TOWNSEND J., PAKE G. E., 1954. — Nature, 174, 689. Citati da MUNDAY e KERKUT.

CORTI A., 1851. — *Recherches sur l'organe de l'ouie des mamifères*. Z. Wis. Zool., 3, 109.

CONTI A., 1961. — *Modificazioni indotte dalla stimolazione acustica sulla distribuzione dell'acetilcolinesterasi nella coclea di cavia*. Arch. It. Otol., 72, 320.

CONTI A., BORGO M., 1963. — *Studio istochimico sul comportamento dell'attività citocromoossidasica nei tessuti ed organi di pertinenza otorinolaringoiatrica. Rilievi istoenzimologici nella cavia*. Arch. It. Otol., 74, 689.

—, 1964. — *Behaviour of cytochrome oxidase activity in the coclea of the guinea-pig, following acoustic stimulation*. Acta Otorinolaryng. (Stock), 58, 321.

CRUICKSHANK C. N. D., HARCOURT S. A., 1963. — *Pigment donation in vitro*. J. Invest. Derm., 41, 183.

FIGGE F. H. J., 1940. — Proc. Soc. Exp. Biol. and Med., 44, 293. Citato da MUNDAY e KERKUT.

FITZPATRICK T. B., SZABO GEORGE, 1959. — *The melanocyte: cytology and cytochemistry*. J. Invest. Derm., 32, 197.

FITZPATRICK T. B., SEIJI M., MC GUGAN A. D., 1961. — *Melanin pigmentation*. New Eng., J. Med., 265, 374.

FRIEDEN E. H., BOZER J. M., 1951. — *Effect of administration of intermedin upon melanin content of skin of Rana pipiens*. Proc. Soc. Exp. Biol. Med., 77, 35.

HOGBEN L. T., WINTON F. R., 1922. — *The pigmentary effector system. 1. Reaction of frog's melanophores to pituitary extracts*. Proc. Roy. Soc. B., 93, 318.

KARKUN J. N., MUKERJI B., 1953. — *Studies on chromatophorotropic hormone of the pituitary gland. Part III. The influence of melanophore hormone upon the synthesis of melanin pigments in the skin of frogs. (Rana tigrina).* Indian J. Med., Res., 41, 467.

KERKUT G. A., EDWARDS M. L., LEACH K., MUNDAY K. A., 1961. — Expetientia, 17, 497. Citati da MUNDAY e KERKUT.

KOIDE Y., HANDO R., YOSHIKAWA Y., 1964. — *Distribution of some oxidizing in the cochlea.* Acta Otolaryng. (Stock.), 58, 344.

KOLMER W., 1927. — *Über die innervation des Tegmentum vasculosum des Vogellabyrinths.* Anat. Anz., 63, 240.

ISHIZAKI Y., BELTER L. F., 1960. — *Melanin deposition in the placenta as a result of skin lesions (Dermatopathic Melanosis of Placenta).* Amer. J. Obstet. Gynec., 79, 1074.

LERNER A. B., SHIZUME R. and BUNDING I., 1954. — *The mechanism of endocrine control of melanin pigmentation.* J. Clin. Endocr., 14, 1463.

LERNER A., MC GUIRE J. S., 1961. — *Effect of alpha and beta melanocyte stimulating hormones on the skin colour of man.* Nature (London), 189, 176.

LUCAE A., 1864. — *Anatomisch-physiologische Beitrage zur Ohreubeilkunde.* Virchows Arch. Path. Anat., 29, 33.

LONGUET-HIGGINS H. C., 1960. — Arch. Biochem. Biophys., 86, 231. Citato da MUNDAY e KERKUT.

MASON H. S., INGRAM D. J. E., ALLEN B., 1960. — Arch. Biochem. Biophys., 86, 225. Citato da MUNDAY e KERKUT.

MIAN E., 1962. — *Proprietà endoatmiche nel melanocita.* Ann. it. Dermat. Clin. Sper., 16, 282.

MITCHELL R. E., 1963. — *The effect of prolonged solar radiation on melanocytes of the human epidermis.* J. Invest. Dermatol., 41, 200.

MUNDAY K. A., KERKUT G. A., 1962. — *Free Radicals in Melanin.* Ann. It. Dermat. Clin. Sper., 16, 310.

QUEVEDO W. C. and GRAHN D., 1958. — *Effect of daily gamma-irradiation on the pigmentation of mice.* Radiat. Res., 8, 254.

RANVIER L., 1889. — *Traitè Technique d'Histologie.* Edit. F. Lavy, Paris.

SACCHI S., 1962. — *Impostazione di qualche problema riguardante alcune recenti acquisizioni sulla melaninogenesi.* Ann. It. Dermat. Clin. Sper., 16, 421.

SIEBENMANN F., BING L., 1907. — *Über der Labyrinth und Hirnbenfund bei einem an retinitis pigmentosa erblindten angehoren Taustummen.* Ztschr. fur Ohrenheilk., 54, 265.

SNELL R. S. and BISCHITZ P. G., 1960. — *The effect of large doses of estrogen and progesterone on melanin pigmentation.* J. Invest. Derm., 35, 75.

—, 1960. — *A study of the melanocytes and melanin in the skin of the immature, mature, and pregnant female guinea-pig.* Z. Zellforsch, 51, 225.

SNELL R. S., — 1962. — *Effect of the melanocyte stimulating hormone of the pituitary on melanocytes and melanin in the skin of guinea-pigs.* J. Endocr., 25, 249.

—,1963. — *The changes produced by Infra Red irradiation in melanin pigmentation of the skin.* Brit. J. Derm., 75, 71.

—, 1963. — *Effect of ultra violet irradiation on melanogenesis.* J. Invest. Derm., 40, 127.

—, 1963. — *Effect of the alfa melanocyte stimulating hormone of the pituitary on mammalian epidermal melanocytes.* J. Invest. Dermat., 41, 337.

SZABO GEORGE, 1954. — *The number of melanocytes in human epidermis.* Brit. Med., J., 1, 1016. /868

—, 1959. — *Quantitative histological investigations on the melanocyte system of the human epidermis.* Pigment Cell Biology p. 99-125. N. Y. Academic Press Inc.

SZENT-GYÖRGYI A., 1957. — « *Bioenergetics* » *Academic. Press.* New York.

YOSHIKAWA Y., MARIMOTO M., 1962. — *Some Oxidizing enzymes in the cochlea.* Ann. of Otorinolaryng., 71, 96.

WASSERMANN H. P., 1963. — *Lymphocytes and the transport of melanin. Observations in the inflammatory response.* J. Invest. Dermatol., 41, 377.

—, 1963. — *An assessment of the phagocytic ability of melanin-labelled L. E. cells and neutrophils in the human skin window.* S. Afr. J. Lab. Clin. Med., 9, 142.

—, 1963. — *Fusion of melanin-labelled macrophages to form giant cells observed in vivo.* J. Invest. Derm., 40, 73.

—, 1964. — *Studies on melanin-labelled cells in the human skin window.* S. Afr. J. Lab. Clin. Med., 10, 76.

—, 1964. — *Inverted neutrophile-lymphocyte ratios in peripheral blood.* A Brief report. Blood., 24, 668.

BIBLIOGRAPHY (Cont.)

—, 1964. — *Doble bodies in Bufo temporaria afflicted with red leg disease.* Nature (London), 202, 816.

—. *The circulation of melanin-its clinical and physiological significance. Review of literature on leucocytic melanin transport.* S. Afr. Med. J. (to be published).

—, 1965. — *Leucocytes and melanin pigmentation. Demonstration of pigmented leucocytes in blood of amphibians, reptiles and normal man.* J. Invest. Derm., **45, 104.**

WHITE L. P., 1958. — Nature, 182, 1427. Citato da MUNDAY e KERKUT.

WOLFF D., 1931. — *Melanin in the inner ear.* Arch. ot Otolaryng., 14, 195.

ZORZOLI G. C. e VERNERONI G., 1950. — *Ricerche istochimiche sul pigmento presente nell'orecchio interno di alcuni mammiferi.* Monit. Zool., 58, 95.